Lipid-soluble inert indicator and radio-active fat in fat absorption studies

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SUMMARY

The determination of fat absorption is best carried out by means of a balance study. In the clinic a fat balance study is as a rule performed by giving the subject a constant diet containing a constant amount of fat, e.g. for eight days. Three days after the beginning of the diet the stool is collected and this is continued for five days. The amount of fat excreted in the stool, subtracted from the amount of fat ingested in the period that stool collection is carried out, is the amount of fat absorbed.

In this method the fat absorption measured is not the net absorption of dietary fat, as the endogenous fat is included in the faecal fat to be subtracted from the amount of fat ingested. Furthermore, this method of performing a balance study is rather laborious.

To meet these objections, the inert indicator method is introduced. A suitable inert indicator and a radio-active fat in a known ratio are given to the subject in one single dose. In a representative stool portion the ratio of these two substances are determined. From these two ratios the percentage of the radio-active fat absorbed can be easily calculated.

As the fat absorption determined in this way refers only to the fat ingested together with the inert indicator, the ratio of which is known, this fat should be discernible from other fat derived from normal food and endogenous fat. For this reason a radio-active fat is used.

In chapter I it is tried to show that the inert indicator method in absorption study in fact employs a modification of the well-known principle of Fick. The inert indicator method described in this thesis makes use of a modification of this principle in which the test diet (test substance and inert indicator) is administered in one single dose.

If the proper inert indicator is used, results obtained with this

method and with the complete collection of the stool are theoretically identical.

The criteria to be fulfilled by a substance as a proper inert indicator in fat absorption studies are:

(1) it should be non-toxic;
(2) it should be quantitatively determinable before and after its passage through the gastrointestinal tract;
(3) it should be unabsorbable;
(4) it should not interfere with the processes of digestion and absorption;
(5) it should mix completely and uniformly with fat and possess the same transit rate through the gastrointestinal tract as the fat.

The best guarantee that criterion number 5 is fulfilled is the lipid solubility of the substance used as inert indicator.

In chapter II a brief survey is given on the substances used as inert indicators in the literature. The most popular are chromic oxide (Cr_2O_3) and PEG (polyethylene glycols with a molecular weight of about 4000). However, there is no substance used as an inert indicator which fulfils all criteria theoretically considered necessary for an inert indicator in fat absorption studies. Criterion number 5 in particular has been difficult to meet.

In chapter III experiments are described whether DC Silicone Fluid 703, which is lipid-soluble, can be used as an inert indicator in fat absorption studies.

DC Silicone Fluid 703 is a diffusion pump fluid designed for extremely high vacuum applications. It is heat-stable, oxidation-resistant, non-corrosive, non-toxic and is chemically inert. It is soluble in lipid solvents such as chloroform, toluene, petroleum ether.

Studies in the literature have shown that DC Silicone Fluid 703 is not toxic. It is found that this substance is not absorbed from the gastrointestinal tract of rats, the recovery in the stool being $96.0 \pm 6.7\,\%$ ($M \pm S.D.$). This is in agreement with the in vitro experiment, in which known amounts of DC Silicone Fluid 703 were added to rat's faeces, extracted and determined. The recovery was $93.8 \pm 4.0\,\%$.

DC Silicone Fluid 703 in the amount used as inert indicator in our experiments (75 mg) has no influence on the absorption of glyceryl-tristearate-1-C^{14} in olive oil. Administered to rats by gastric tube with and without DC Silicone Fluid 703, the absorption of the radio-active fat was 89.2 ± 3.6 % and 90.3 ± 4.6 %, respectively.

The experiments carried out with rabbits fitted with an ileo-coecal cannula to demonstrate that DC Silicone Fluid 703 has the same transit rate through the digestive tract as the fat, did not yield satisfactory results. This is probably due to the fact that the fat absorption when considered within very short intervals (15-45 minutes) is not constant.

The absorption of glyceryl-tristearate-1-C^{14} in olive oil in rats, determined by means of DC Silicone Fluid 703 as inert indicator and by means of the complete collection of the stool, was 88.8 ± 4.0 % and 89.2 ± 3.6 %, respectively.

From the results obtained from the experiments described in chapter III, it may be concluded that DC Silicone Fluid 703 may be considered a good indicator for use in fat absorption studies in the rat.

Chapter IV gives a brief survey on the literature concerning the radio-iodinated triolein (RIT). In the literature RIT was used for the determination of the fat absorption, using as parameters blood radioactivity, urine radioactivity and faecal radioactivity. There are many objections to the use of blood and urine radioactivity as a parameter for fat absorption, as its radioactivity after fixed time intervals depends on the rate and amount of absorption, utilization, storage and extravascular sequestration of fat, completeness of thyroid block for iodine, the renal clearance of free iodine, the fat load and dosing procedure and the rate of gastric emptying. These factors have no influence on faecal radioactivity. The determination of RIT in the stool can be carried out without difficulty, as the gamma rays emitted by the I^{131} isotope are easily assessed.

The results reported on the use of RIT in fat absorption studies are controversial. The papers by TUNA et al. (1963) and KENNEDY and KINLOCH (1964) reporting that commercial RIT preparations contained 30-60 % radio-active impurities, may explain at least part of these contradictory results. Because RIT can be easily determined in the stool, it seemed worth while, in the light of the papers by TUNA et al. and KENNEDY and KINLOCH, to investigate whether purified RIT is a good substitute for natural triolein, in other words whether RIT can be used as a test fat in fat absorption studies.

As C^{14} labelled triolein is considered identical with natural triolein, RIT was compared with glyceryl-trioleate-C^{14}. This substance is not used clinically, as its application to human subjects is considered rather hazardous, because of the long physical half life (5568 years) of the carbon isotope. According to GRENIER et al. (1964), however, the overall biological half life of C^{14} labelled fat is only 17-35 days. Another objection to the use of C^{14} labelled fat is that the low energy beta emission of the C^{14} isotope is difficult to determine in the stool.

Results obtained from experiments described in chapter V show that there was no difference in the absorption of RIT and glyceryl-trioleate-C^{14} in olive oil in the rat, determined by the radioactivity in the stool. The absorption of RIT in olive oil was 91.7 ± 7.6 % and of C^{14} labelled triolein in olive oil 91.6 ± 9.8 %.

In the thoracic duct's lymph of the rat RIT was found massively deiodinated, up to 20-30 %. No deiodination, however, was found in the stomach, small intestine, coecum and colon and in the stool. But it appeared that the same massive deiodination as was found in the thoracic duct's lymph, was also present in the small intestinal wall. These results suggest that RIT was not deiodinated in the intestinal lumen. In the intestinal wall, massive deiodination of the RIT occurred and the result of it persisted in the thoracic duct's lymph.

From the above it may be concluded that RIT can be used as a test fat in fat absorption studies in the rat, if faecal radioactivity is used as the index for fat absorption.